Guidelines for Environmental and Chill Water/Brine Sampling for *Listeria*

Introduction

This protocol details possible procedures for environmental sampling for *Listeria* species. It provides guidelines for selection of environmental sample sites that may be targeted for FSIS investigations. This protocol also includes the FSIS laboratory procedure for initiation of analysis of environmental samples for *Listeria monocytogenes*. The FSIS methodology for *L. monocytogenes* (MLG Chapter 8, Rev. 2) may be adapted for the detection of *Listeria* species. FSIS acknowledges that alternative sampling and laboratory methodology may be equivalent or more appropriate for certain applications. Therefore, this document is a guideline, not a directive.

Each meat or poultry plant presents a unique environment. Therefore, the selection of appropriate sample sites and the number of samples taken must be determined on a case-by-case basis. Sampling should be primarily representative of product contact surfaces with other sampling performed as appropriate. Although this protocol is intended for investigational purposes, it may be used to develop routine environmental sampling conducted by meat and poultry processing plants.

Personnel involved should be experienced in environmental sampling and cognizant of microbiological issues that could adversely affect the integrity of the sample. These personnel should also be knowledgeable in the function of plant equipment and the typical flow of product through a plant environment.

Selection of environmental sampling sites

As a general rule, environmental sampling should be performed approximately mid-shift. For safety, have plant personnel shut down machinery for the time required for sampling. Pre-operational sampling should also be performed to assess efficacy of cleanup and sanitization.

Every processing line offers unique environments for potential harborage of *Listeria* species. Therefore, it is critically important that the inspector/sampler specifically assess potential contamination sites for each product line and make a list of priority sites prior to sampling.

L. monocytogenes may contaminate a food-processing environment in a variety of areas, some of which are more relevant to the potential contamination of finished product. Therefore, an example list of primary and secondary sampling sites is prioritized below. This list may not include important sampling sites unique to a particular production line.

The presence of *Listeria* species on sampled environmental sites may warrant testing of finished product. Refer to the FSIS publication "*Listeria* Guidelines for Industry" (May, 1999) for guidance on routine and follow-up environmental and product sampling. The recently published "Guidelines to Prevent Post-Processing Contamination from *Listeria monocytogenes*" (Tompkin *et al.*, Dairy, Food and Environmental Sanitation, **19**(8):551-562) provides industry perspective on potential environmental sampling sites and prevention of *Listeria* contamination problems.

Primary environmental sampling site examples (product contact surfaces)

Food residues and moisture promote proliferation of *Listeria* species. Historically, *Listeria* contamination has been introduced to the product after the cooking step (i.e. lethality treatment). Therefore, post-process product contact surfaces are important environmental sampling sites. Contamination of plastic, metal and other surfaces that come in contact with

unpackaged product indicate a significant contamination risk for product. Product contact surfaces may include but are not necessarily limited to:

1.) Conveyor belts that contact unpackaged post-process product
2.) Table and counter tops that contact unpackaged post-process product
3.) Peeler apparatus
4.) Slicing equipment
5.) Packaging equipment
6.) Chill water and/or brines that directly contact unpackaged product*
7.) Any difficult-to-clean product contact surface areas along the line
8.) Product contact surface sites soiled by food residue

* Non-chlorinated chill water/brine is more likely to be contaminated by *Listeria* spp. than chlorinated chill water/brine.

Secondary environmental sampling site examples (non-product-contact surfaces)

Environmental surfaces that do not directly contact unpackaged product include, but are not necessarily limited to:

1.) Chill water and brines that contact packaged product
2.) Condensation pans, drip pans and reservoir tanks
3.) Exterior and interior surfaces of chill tanks
4.) Interior surfaces of refrigerators, freezers and cold rooms
5.) Conveyor system surfaces that do not contact unpackaged post-process product including the belt, hollow rollers and drive chains
6.) Table and counter top surfaces that do not contact unpackaged post-process product
7.) Any difficult-to-clean non-product contact areas along the line
8.) Non-product contact surfaces soiled by food residue
9.) Handled areas such as levers, machine/light switches and door frame/knobs
10.) Overhead areas including overhead conveyor belts
11.) Wet cart wheels

The following areas have been frequently shown to harbor *Listeria* species but do not necessarily indicate food product contamination:

1.) Floor and other drains
2.) Other wet areas on the floor including the underside of floor mats
3.) Trash containers

Supplies required for environmental sampling

1.) Sterile non-bactericidal cellulose specimen sponge in a sterile Whirl-Pak® bag or equivalent. Although smaller Whirl-Pak® bags are acceptable, a 55 oz. 7.5" x 12" bag provides additional volume to accommodate laboratory analysis.
2.) Empty Whirl-Pak® bag or equivalent, 7.5" x 12", 4 mil thick or of sufficient size to double bag the above.
3.) Sterile disposable latex, vinyl or nitrile gloves.
4.) Dey-Engley (DE) neutralizing broth. This must be stored in a 2-8◦ C refrigerator.
5.) Felt-tip marker with waterproof black ink.
6.) Basket or equivalent (e.g. "tote") to hold bags upright during manipulations.
7.) Sample shipper with pre-frozen refrigerant packs and cardboard spacers.

8.) Sterile 50-ml ladle with handle or equivalent device (e.g. pipette) for aseptic sampling of solutions (e.g. chill water/brine).
9.) Sterile specimen cup, 100-150 ml volume, or equivalent sterile plastic vessel with watertight screw cap (i.e. for sampling chill water/brine).
10.) Steel tape measure and/or appropriately-sized template(s).

Environmental surface sampling procedure

1.) Wash and sanitize your hands to the mid-forearm. Use clean disposable paper towels for drying your hands. If feasible, pre-determine (e.g. using a tape measure or template) and mark the area to be sampled as described in step 8 below.
2.) Using a felt-tip black permanent marker, label the bag with appropriate sample information.
3.) Pull off the top of the Whirl-Pak® or equivalent bag along the perforation. Using the tabs on both sides of the wired band, pull gently to open the bag. Place the opened bag upright in the basket/tote. Do not remove the sponge.
4.) Aseptically pour into the bag 9-10 ml or sufficient volume of DE neutralizing broth to hydrate the sponge. Be careful not to touch the opening of broth container to any non-sterile surface before or during this transfer.
5.) Massage the sponge through the outside of the bag to facilitate absorption. From the outside of the bag, push the sponge to the upper portion of the bag. While pushing the sponge up from the bottom of the bag, squeeze excess DE broth from the sponge back into the bag. The sponge should be moist but not dripping wet. Position the sponge vertically in the bag toward the opening and place the bag containing the sponge upright in the basket/tote. Refer to Section 6, steps 7-9, of the FSIS guidebook Self-Instruction Guide for Collecting Raw Meat and Poultry Product Samples for *Salmonella* Analysis (FSIS Directive 10,230.5 and 10,230.5, Amendment 1) for an illustrated guide on the manipulation of the sponge from outside of the bag.
6.) Aseptically place a sterile glove upon the hand you will use for swabbing. Refer to Section 4 of Self-Instruction Guide for Collecting Raw Meat and Poultry Product Samples for *Salmonella* Analysis for an illustrated guide in the proper use of sterile disposable gloves. Do not touch any non-sterile surface (e.g. clothes, skin, counter tops, etc.) with the outside surface of the sterile glove. The other hand can be left ungloved for manipulation of non-sterile surfaces and materials.
7.) Using the ungloved hand, use the tab on the outside to hold the opened bag. Using the gloved hand, carefully remove the sterile moistened cellulose sponge. Do not touch the exterior surface of the bag with the sterile glove or sponge.
8.) Using even and firm pressure, push the sponge in one direction across the desired area of the environmental surface 10 times vertically, then 10 times horizontally. Sampling of large flat surfaces (e.g. floor, table tops, conveyor belts) should cover a 2 ft. by 3 ft. (6 sq. ft.) area or all available area deemed appropriate. For flat surfaces, use a tape measure or template to measure/mark the area to be sampled (i.e. 2 ft. by 3 ft. = 6 sq. ft. or all appropriate and available area). Sampling of more complex surfaces (e.g. equipment) should be sufficient to cover all representative areas roughly equivalent to at least 6 sq. ft. (or all available area if less than 6 sq. ft.) including "nooks and crannies". Carefully replace the sample sponge in the Whirl-Pak® or equivalent bag. Be careful not to touch the exterior of the bag or any other non-sterile surface with the sponge.
9.) Remove the contaminated glove and discard. Squeeze as much air out of the bag as possible. Roll the top of the bag over several times until it is folded all the way down to the sponge. Fold in the tabs to lock the fold in place. Place the sponge bag inside another empty Whirl-Pak® or equivalent bag and seal as before. Both bags must be tight enough to provide both a leakproof seal and minimal airspace during shipment of the moistened sponge.

10.) As soon as possible, place the double-bagged sponge inside an insulated sample shipper, preferably pre-chilled, with two pre-frozen gel packs. Shippers used for FSIS HACCP samples can accommodate up to 20 sponge samples.
11.) Ship the chilled samples by overnight service to the laboratory for analysis.

Liquid sampling procedure for chill water/brine

1.) Wash and sanitize hands as above.
2.) Label (e.g. with a felt tip marker) all sterile specimen cups/bottles to be used for sampling.
3.) Aseptically open two specimen cups. Carefully open sterile ladle package from handle end. Aseptically remove the sterile 50-ml ladle (or equivalent sterile measuring device) from the sterile packaging (i.e. pull ladle out by the handle). Do not touch the scoop end of the ladle against any non-sterile surface, including the exterior of the ladle packaging.
4.) Scoop/pipette the chill water/brine from the tank with the sterile end (i.e. the scoop part) of the ladle/pipette only. Aseptically transfer one scoopful or 45-55 ml of sample into each of the two opened specimen cups/bottles. Note the volume placed into each cup/bottle.
5.) To neutralize chlorination and other disinfectants, aseptically add an equivalent volume of DE (i.e. 45-55 ml) to each sample collected. The amount of sample and DE should be equal and the volumes of each should be noted. The chlorine concentration of the chill water/brine, if known, should be noted.
6.) Tightly cap the specimen cups/bottles, gently mix, and then place them into individual Whirl-Pak® or equivalent bags. Seal the bags (but do not whirl) and place them immediately in an insulated sample shipper, preferably pre-chilled, with at least two pre-frozen gel packs.
7.) Ship by overnight service to the laboratory on the day of sampling as above.

Laboratory analysis of environmental samples for *L. monocytogenes*

The FSIS method for detection and specific identification of *L. monocytogenes* is detailed in the FSIS Microbiology Laboratory Guidebook (MLG), 3rd Edition, 1998, Chapter 8, Revision 2. The current revision is posted on the FSIS web site at http://www.fsis.usda.gov. Check the web site periodically for updated information.

The MLG Chapter 8, Revision 2 method is used by FSIS laboratories and is specific for *L. monocytogenes*. Alternatively, non-FSIS laboratories may opt to employ rapid screening procedures to adapt this or other methods for detection of *Listeria* species. Laboratories should use validated methods or methods published in peer-reviewed scientific journals to detect *Listeria* species in environmental samples.

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